Products from the Thermal Degradation of Some Naturally Occurring Materials

E. Barr Higman, Irwin Schmeltz, and William S. Schlotzhauer

A number of carbohydrates, proteins, and amino acids were each pyrolyzed at 840° C under nitrogen. Products generated fell into two categories, those commonly present in all pyrolyzates and those singularly characteristic of the material pyrolyzed. Aromatic hydrocarbons were present in all pyrolyzates; absent from the pyrolyzates of casein and collagen was the biologically active hydrocarbon, benzo[*a*]pyrene. Both proteins and carbohydrates produced phenols. Proteins also gave rise to a series of pyridine bases, quinoline, indole, pyrrole, nitriles, and aniline. The amino acids, proline and glycine, generated nitrogen-containing products similar to those obtained from the proteins examined.

During studies designed to establish precursor-product relationships between components of tobacco smoke and compounds present in tobacco leaf, we subjected several proteins, carbohydrates, and amino acids to thermal degradation at 840° C, the approximate burn temperature of a cigarette. Data obtained and presented herein should shed some light on chemical transformations occurring in this temperature region. In addition, the reported physiological effects of a number of polynuclear aromatic hydrocarbons and their presence in some foods and other commodities (Howard and Fazio, 1969) have spurred interest into their mode of origin. Much evidence indicates that high temperatures may play a role in their formation (Gilbert and Lindsey, 1957; Jones and Schmeltz, 1969; Schlotzhauer and Schmeltz, 1968, 1969).

In the present report, compounds produced by exposing proteins, amino acids, and carbohydrates to high temperatures are identified. Some of the products generated are shown to be peculiar to certain types of naturally occurring substances, whereas others are more general.

EXPERIMENTAL

Materials. The proteins, casein, collagen, and β-lactoglobulin A were obtained from colleagues. The carbohydrates, cellulose (Brown, Boston), fructose and glucose (Sigma Chemical, St. Louis), and the amino acids, proline (Merck, Rahway) and glycine (Eastman-Kodak, Rochester), were used as obtained.

Pyrolytic Techniques. Pyrolyses were performed in a Vycor tube (2.6 cm o.d. × 35 cm length) positioned horizontally inside a Lindberg Hevi-Duty Furnace, maintained usually at 840 ± 10° C. A Chromel-Alumel thermocouple was used to monitor pyrolysis temperature. The pyrolysis tube (packed with quartz chips) was flushed with nitrogen (60 ml per min).

Approximately 1 g solid samples were placed in ceramic boats, which were placed in the preheated Vycor tube immediately prior to pyrolysis. Volatile, condensable products were collected in a series of three traps, the first cooled in an ice-water mixture, and the second and third in dry ice-acetone. The remaining product stream passed through a gas scrubber containing ether and 5% aqueous NaOH.

Fractionation of Pyrolyzate. The pyrolyzate was extracted with ether and 5% aqueous NaOH; the two washings were mutually extracted, yielding an ether solution containing neutrals and bases and an aqueous solution containing acids and phenols. Successive adjustments of the aqueous alkaline extract first to pH 6.8 and then to pH 1.5 with 25% aqueous H_2SO_4 liberated phenolic and acidic products, respectively, which were taken up in ether. The original ether solution above, containing bases and neutrals, was extracted with 1N HCl to remove bases which were reextracted with ether after alkalinization of the HCl extract. Ether solutions of neutrals, bases, acids, and phenols were thus ultimately obtained which, after drying and concentrating, were suitable for gas chromatographic analysis. Residue weights are given in Table I. These were obtained by carefully evaporating aliquots of the respective ether solutions to dryness (constant weight) under vacuum.

Gas Chromatographic Analysis. Pyrolyzates were monitored by gas chromatography on a Varian Aerograph Model 200 instrument equipped with dual stainless steel columns (5 ft × 0.25 in. o.d. 20% SE-30 on 60/80 mesh Chromosorb W, or 10 ft × 0.25 in. o.d. 20% SE-52 on 60/80 mesh Chromosorb W). Column temperature was programmed from 70° to 250° C at 6° C per min (SE 30 column) for analysis of carbohydrate (Figure 1) and protein (Figures 2–4) pyrolyzates, and at 8° C per min (SE-52 column) for analysis of amino acid pyrolyzates. Column temperature was maintained at 250° C until elution of high-boilers was complete. Detector and injector temperatures were set at 300° C, and helium flow at 75 ml per min.

Gas chromatographic peak effluents were identified, where possible, by comparing their spectra (infrared, ultraviolet, and/or mass) with those of known compounds, and by coinjection studies. Relative yields of pyrolytic products were determined by measuring their corresponding peak areas on the gas chromatograms by the method of triangulation. These yields are presented in Tables II and III as percentages of the various fractions.

Determination of Benzo[*a*]pyrene Levels. Benzo[*a*]pyrene was determined by a method combining thin-layer chromatography and ultraviolet spectrophotometry (Schmeltz *et al.*, 1964).

RESULTS AND DISCUSSION

Carbohydrates. Much of the previous work on carbohydrate pyrolysis has been done at relatively low temperatures (<400° C) and has resulted primarily in the identification, in pyrolyzates, of furans (including furfural and its derivatives), aliphatic aldehydes and ketones, simple aromatic hydrocarbons, and phenols (Fagerson, 1969). Anhydrohexoses have also been reported to be pyrolytic products of carbohydrates (Gardiner, 1966; Heyns *et al.*, 1966).

Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pa. 19118

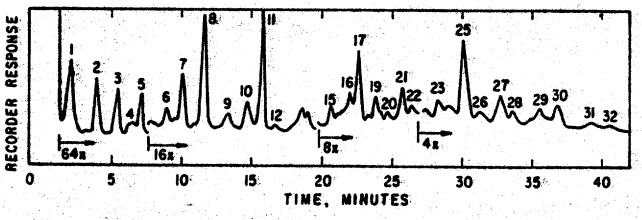

Figure 1. Gas chromatogram showing neutral products from pyrolysis of fructose

1. Benzene 2. Toluene 3. Furfural 4. Ethylbenzene 5. Styrene (xylenes) 6. 5-Methylfurfural 7. *p*-Methylstyrene 8. Indene 11. Naphthalene 13 and 14. Alkylnaphthalenes 15. Biphenyl 16. Acenaphthylene 19. Fluorene 20. Dibenzofuran 21. Phenanthrene (anthracene) 22. Alkylphenanthrene (alkylanthracene) 23. Fluoranthene 25. Pyrene 31. Benzofluorene. Other peaks were not identified

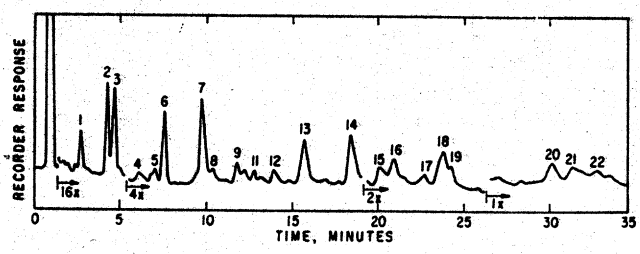

Figure 2. Neutral products from pyrolysis of casein

1. Benzene 2. Pyrrole 3. Toluene 6. Styrene 7. Benzonitrile 9. Indene 10. *o*-Tolunitrile 11. *m*-Tolunitrile 13. Naphthalene 14. Indole 18. Fluorene. Other peaks were not identified

Table *I*. Weights of Fractions (in mg per gram pyrolyzed) Obtained from Pyrolysis

	Cellulose	Glucose	Fructose	Casein	Collagen	Proline
Neutrals		17.7	19.4	413.7	354.0	47.6
Acids		14.4	5.6	4.1		9.4
Phenols		10.4	6.2	71.5	20.7	10.7
Bases				225.9	257.5	16.4
Ash[a]	29.9	16.1	55.8	144.4	94.3	
Total		58.6	87.0	859.6	726.5	

[a] Carbonaceous residue left in combustion boat after pyrolysis.

At 840° C, the products from carbohydrate thermolysis (Table II) comprised a very complex mixture (Figure 1). The products included the condensed ring hydrocarbons, simpler aromatic hydrocarbons, furfurals, and phenols (Table II). Glucose and fructose, but not cellulose, yielded furfural and 5-methyl furfural at the high temperature of pyrolysis. The stability of these two compounds, however, has been noted previously (Fagerson, 1969). New compounds shown to arise from pyrolysis of sugars include styrene, *p*-methylstyrene, indene, and dibenzofuran. Methyl and dimethylphenols, in addition to phenol, were noted in the pyrolyzates from cellulose, glucose, and fructose. The pyrolytic formation of phenols from the carbohydrates, pectin, cellobiose, glucuronic, and polygalacturonic acid has been observed (Schlotzhauer *et al.*, 1967). In tobacco smoke, the presence of phenols along with polynuclear aromatic hydrocarbons, especially benzo[*a*]pyrene, is physiologically significant (Wynder and Hoffmann, 1967).

Benzo[*a*]pyrene levels were determined in the various pyrolyzates. The amounts from pyrolysis of glucose, fructose, and cellulose are shown in Table IV. The value we obtained

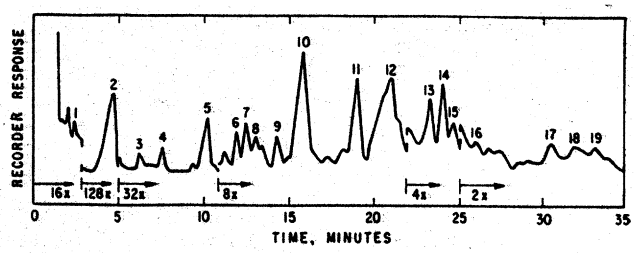

Figure 3. Neutral products from pyrolysis of collagen

1. Benzene 2. Pyrrole (toluene) 4. Styrene 5. Benzonitrile 6. Indene 7. *o*-Tolunitrile 8. *m*-Tolunitrile 10. Naphthalene 12. Indoles (mixture). Other peaks were not identified

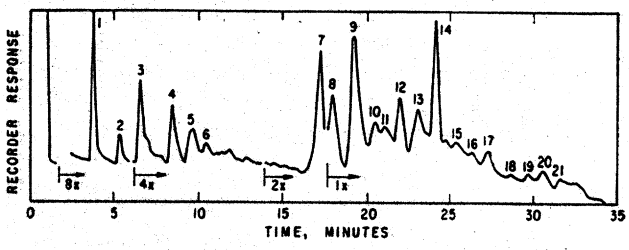

Figure 4. Bases from pyrolysis of collagen

1. Pyridine 2. α-Picoline 3. β-,γ-Picoline 4. 3-Vinylpyridine 5. Aniline 7. Quinoline 8. Isoquinoline. Other peaks were not identified

Table II. Percent Distribution of Products from Pyrolysis (840° C, N_2)

Neutrals[a]	Cellulose	Glucose	Fructose
Benzene	25.7	17.6	27.4
Toluene	24.9	14.6	14.2
Furfural		7.4	12.5
Ethylbenzene	2.2	4.7	3.5
Styrene, xylenes[b]	6.1	7.1	7.0
5-Methylfurfural		2.3	1.0
p-Methylstyrene	3.7	4.6	3.7
Indene	6.0	6.3	10.6
Naphthalene	10.3	8.0	5.7
Alkylnaphthalene	4.3	3.3	2.3
Biphenyl			0.4
Acenaphthylene	1.3	1.1	0.4
Fluorene	1.8	0.4	0.5
Dibenzofuran			0.2
Phenanthrene, anthracene[b]	0.9	0.8	0.8
Alkylphenanthrene, alkylanthracene[b]			0.2
Fluoranthene			0.03
Pyrene	2.1		0.13
Phenols[a,c]			
Phenol	67.5	59.6	77.9
o-Cresol	8.4	10.6	4.8
m-Cresol, *p*-cresol[b]	12.4	11.7	4.3
Xylenol	4.2	3.9	2.3

[a] In order of gas chromatographic retention times. [b] Inseparable by glc method used. [c] Formic and/or acetic acid were found in the acid fraction of the pyrolyzates from glucose and fructose.

from glucose pyrolysis is considerably higher than that obtained by Masuda *et al.* (1967); the difference may reflect variations in pyrolysis conditions, including temperature.

The mode of formation of a number of the products from carbohydrate pyrolysis has been the subject of several reports (Fagerson, 1969). However, the pathway from sugars to aromatic hydrocarbons, especially the condensed ring types, remains obscure.

Table III. Percent Distribution of Products from Pyrolysis (840° C, N_2)

Neutrals[a]	Casein	Collagen	Proline[b]
Benzene	9.0	0.6[c]	2.8
Pyrrole, toluene[d]	39.7[e]	64.3[e]	57.1[e]
Styrene, xylenes[d]	5.8	2.2	
Benzonitrile	9.7	6.8	0.7
Indene	1.6	0.8	
o-Tolunitrile	1.2	1.2	0.6
m-Tolunitrile	1.0	1.0	0.2
Naphthalene	5.5	5.5	
Indole	6.8	7.2[f]	36.6[g]
Fluorene	2.8		
Bases[a]			
Pyridine	23.0	26.5	10.1
α-Picoline	8.5	11.3	2.5
β- and/or γ-picoline[d]	7.6	10.0	2.2
3-Vinylpyridine	7.3	6.3	
Aniline	7.8	5.7	1.5
Quinoline	13.4	7.6	2.0
Isoquinoline	3.6	3.2	10.2
Phenols[a,h]			
Phenol	35.3	19.6	
o-Cresol	1.0		
m-Cresol, *p*-cresol[d]	26.8	8.1	
Ethylphenol	0.5	2.3	
Xylenol	4.8	2.9	

[a] In order of gas chromatographic retention times. [b] Glycine on pyrolysis yielded the following, identified tentatively: benzene, pyrrole (toluene), benzonitrile, indene, *o*-tolunitrile, *m*-tolunitrile, naphthalene, indole, fluorene, pyridine, α-picoline. [c] Some benzene lost due to volatility. [d] Inseparable by glc method used. [e] Predominantly pyrrole which was also found in phenolic fraction of proline pyrolyzate. [f] Mixture of indoles. [g] Mixture of indole and other compounds. See text. [h] Formic and/or acetic acid were found in the acid fraction of the pyrolyzate from casein.

Table IV. Levels of Benzo[*a*]pyrene (BaP)[a] Produced on Pyrolysis

Substance Pyrolyzed	μg BaP/g Pyrolyzed
Glucose	47.5
Fructose	98.4
Cellulose	288.8
Collagen	...[b]
Casein	...[c]
Proline	...[d]

[a] Determined by ultraviolet absorption following thin-layer chromatography (Schmeltz *et al.*, 1964). [b] Weak absorption bands at 365 and 385 mμ—possibly a trace of BaP. [c] Absorption bands at 320 and 336 mμ—possibly a benzocarbazole, and 358 and 376 mμ, indicative of a dibenzacridine. [d] Absorption bands at 275, 288, 294, 321 and 336 mμ—characteristic of a benzocarbazole.

Proteins (and Amino Acids). Proteins have been previously subjected to pyrolysis studies, primarily via the technique of pyrolysis-gas chromatography. Such studies, however, merely establish "fingerprint" patterns for different proteins and amino acids (Perry, 1968; Winter and Albro, 1964), with questionable reproducibility and without providing much data on pyrolyzate composition.

Sufficient quantities of casein and collagen were pyrolyzed in the present study to permit collection of the pyrolyzate produced and characterization of the major components. In both cases, as can be seen by the chromatograms obtained (Figures 2–4), the product mixtures were exceedingly complex, the neutral and basic compounds from each pyrolyzate accounting for more than 60 peaks.

Products from the proteins consisted of aromatic hydrocarbons, their nitrogen-containing analogs, nitriles, aniline, and phenols (Table III). Like most organic materials subjected to high temperatures, the two proteins examined produced aromatic hydrocarbons. Unlike most organic materials, however, including carbohydrates, the proteins produced little, if any, benzo[*a*]pyrene (Table IV), although ultraviolet spectral studies indicated the possible presence of nitrogen containing polycyclic compounds such as benzocarbazoles and acridines.

Many of the products in the protein pyrolyzates bear structural relationships to amino acid units comprising the original proteins. These relationships are suggestive of pyrolytic pathways from "precursor to product." Pyrrole, for example, a major constituent of the protein pyrolyzates, probably originated from proline present in the proteins, the pathway proceeding via decarboxylation and dehydrogenation. Moreover, pyrrole was observed to be the predominant product when proline itself was pyrolyzed (Table III).

Pyrolysis of tryptophan reportedly results in the generation of indole, alkyl indoles, and quinoline as major products (Patterson *et al.*, 1969; Rodgman and Cook, 1962) and their presence in the casein pyrolyzate could be attributed, therefore, to tryptophan moieties in the protein molecule. On the other hand, collagen contains no tryptophan (West and Todd, 1961), and the indicated presence of indole and quinoline in the pyrolyzate from this protein would require another explanation. There is evidence, however, for the pyrolytic formation of indoles (and quinoline) from amino acids other than tryptophan (Patterson *et al.*, 1969). In the present study, moreover, indole was apparently produced during the pyrolysis of proline. When gas chromatographed, it eluted with a number of other compounds in a peak that accounted for 37% of the neutral fraction of the proline pyrolyzate, and was therefore only tentatively identified on the basis of mass and ultraviolet spectral characteristics and retention data. Other compounds possibly eluting with indole, as indicated by mass and infrared spectral data, included a vinyl pyrrole and a nitrile. In addition, quinoline, benzene, pyridine bases, and other compounds were produced as a result of proline pyrolysis (Table III). Although it is difficult to visualize the pathway from proline to indole via pyrolysis, a study by Patterson *et al.* (1968) on the pyrolysis of pyrrole, with indole as a major product, may be relevant, indicating that pyrrole is an intermediate in the "proline to indole" pathway. The route from tryptophan to indole (or methyl indole), on the other hand, lends itself to more rational speculation; in this case, the pathway would involve rupture of the tryptophan side chain, either adjacent to or one carbon removed from the ring. Similarly, the formation of quinoline from tryptophan would necessitate side chain participation in a ring enlargement mechanism.

The formation of simple pyridine bases, nitriles, aniline, and aromatic hydrocarbons as a result of protein pyrolysis is again difficult to rationalize mechanistically, and studies of pyrolysis of individual amino acids may be helpful. Hurd and Simon (1962) attempted to elucidate pyrolytic pathways from pyridine and methyl pyridines to nitriles, benzene, and other products, and have suggested a number of mechanistic possibilities which may be applicable to the present problem.

Phenols present in the protein pyrolyzates could also be traced to certain amino acids residing in the protein. *p*-Cresol, for example, could arise from tyrosine (rupture of the side chain one carbon removed from the aromatic ring), and the other phenols from secondary decomposition or rearrangement of the *p*-cresol produced initially. At high temperatures, most reaction schemes can be considered energetically feasible.

In addition to the above proteins, we pyrolyzed β-lactoglobulin A and, following a limited study of the pyrolytic products, identified indole, methyl indoles, phenol, and *p*-cresol. We also noted a number of ninhydrin-positive products which were not common amino acids, and were not amenable to identification.

In the present study, we also pyrolyzed glycine. Identification of the products in this case was based solely on retention data. Tentatively, the products as shown by their chromatograms bore close resemblance to those obtained from proline pyrolysis (Table III).

Related Results From Fatty Substances. Pyrolysis of a number of fatty materials, including dotriacontane, stearic acid, methyl linoleate, β-sitosterol, and others, was undertaken in previous studies (Schlotzhauer and Schmeltz, 1968, 1969). As expected, at 860° C, these substances gave rise to pyrolyzates generally characterized by the presence of aromatic hydrocarbons such as benzene, alkylbenzenes, styrene, and condensed ring systems (indene, naphthalene, pyrene, benzo[*a*]pyrene, and others). β-Sitosterol produced relatively large amounts of phenanthrene and naphthalene consistent with the internal phenanthrene skeleton characteristic of all sterols.

When dotriacontane and stearic acid were pyrolyzed at a lower temperature (650° C), the resulting pyrolyzates no longer contained a preponderance of aromatic hydrocarbons. In preliminary studies, the pyrolyzates in both cases appeared instead to consist of homologous series of monoalkenes, on the basis of gas chromatographic and mass spectral data, confirming previous reports (Holman *et al.*, 1966).

Concluding Remarks. The study described confirms the expectation that most, if not all, organic material will produce polynuclear aromatic hydrocarbons on exposure to high temperatures. Significantly, many types of compounds, including carbohydrates and proteins, on pyrolysis give rise to phenols as well. Certain nonvolatile, naturally-occurring organic acids (Schmeltz *et al.*, 1967), polyphenols (Jones and Schmeltz, 1968; Schlotzhauer *et al.*, 1967), lignin (Schlotzhauer *et al.*, 1967), and a tobacco additive, menthol (Schmeltz and Schlotzhauer, 1968) are also precursors of phenols in high temperature processes. Sodium acetate on pyrolysis has been shown to produce relatively high yields of 3,5-xylenol (Schmeltz and Schlotzhauer, 1969).

The proteins and amino acids that we pyrolyzed produced a series of pyridine bases. Whether such is the case for proteins and amino acids in general remains to be determined. The tobacco alkaloid, nicotine, gives rise to a similar series of pyridine bases on pyrolysis (Schlotzhauer and Schmeltz, 1967).

ACKNOWLEDGMENT

The authors thank Calvin J. Dooley and Stanley F. Osman for obtaining the mass spectral data relative to the findings presented herein. They also thank Edward F. Mellon and Helen A. Gruber, and Norbert J. Hipp for providing the proteins, collagen, and casein, respectively. Edwin B. Kalan and Rae Greenberg provided the β-lactoglobulin A.

LITERATURE CITED

Fagerson, I. S., J. AGR. FOOD CHEM. **17**, 747 (1969).
Gardiner, D., *J. Chem. Soc. (C)* 1473 (1966).
Gilbert, J. A. S., Lindsey, A. J., *Brit. J. Cancer* **11**, 398 (1957).
Heyns, K., Stute, R., Paulsen, H., *Carbohydrate Res.* **2**, 132 (1966).
Holman, R. T., Deubig, M., Hayes, H., *Lipids* **1**, 247 (1966).
Howard, J. W., Fazio, T., J. AGR. FOOD CHEM. **17**, 527 (1969).
Hurd, C. D., Simon, J. I., *J. Amer. Chem. Soc.* **84**, 4519 (1962).
Jones, T. C., Schmeltz, I., *Chem. Ind.* 1480 (1968).
Jones, T. C., Schmeltz, I., *J. Org. Chem.* **34**, 645 (1969).
Masuda, Y., Kazuko, M., Kuratsune, M., *Gann* **58**, 69 (1967).
Patterson, J. M., Baedecker, M. L., Musick, R., Smith, W. T., Jr., *Tobacco Sci.* **13**, 26 (1969).
Patterson, J. M., Tsamasfyros, A., Smith, W. T., Jr., *J. Heterocycl. Chem.* **5**, 727 (1968).
Perry, S. G., *Advan. Chromatogr.* **7**, 221 (1968).
Rodgman, A., Cook, L. C., *Tobacco Sci.* **6**, 176 (1962).
Schlotzhauer, W. S., Schmeltz, I., *Beitr. Tabakforsch.* **4**, 176 (1968).
Schlotzhauer, W. S., Schmeltz, I., *Beitr. Tabakforsch.* **5**, 5 (1969).
Schlotzhauer, W. S., Schmeltz, I., *Tobacco Sci.* **11**, 89 (1967).
Schlotzhauer, W. S., Schmeltz, I., Hickey, L. C., *Tobacco Sci.* **11**, 31 (1967).
Schmeltz, I., Hickey, L. C., Schlotzhauer, W. S., *Tobacco Sci.* **11**, 52 (1967).
Schmeltz, I., Schlotzhauer, W. S., *Chem. Comm.* 681 (1969).
Schmeltz, I., Schlotzhauer, W. S., *Nature* (London) **219**, 370 (1968).
Schmeltz, I., Stedman, R. L., Chamberlain, W. J., *Anal. Chem.* **36**, 2499 (1964).
West, E. S., Todd, W. R., "Textbook of Biochemistry," 3rd ed., p. 280, MacMillan, New York, 1961.
Winter, L. N., Albro, P. W., *J. Gas Chromatogr.* **2**, 1 (1964).
Wynder, E. L., Hoffmann, D., "Tobacco and Tobacco Smoke," p. 231, Academic Press, New York, 1967.

Received for review February 3, 1970. Accepted May 13, 1970. Presented at the Symposium on Air, Water, and Waste Control, Fifth Middle Atlantic Regional Meeting, American Chemical Society, University of Delaware, April, 1970. Mention of commercial products does not imply their endorsement by the Department over similar products not mentioned.